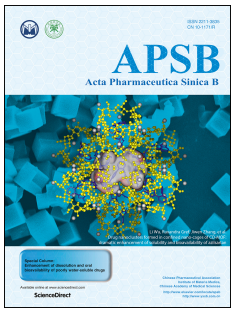
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.



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PII: S2211-3835(20)30552-9

DOI: https://doi.org/10.1016/j.apsb.2020.04.008

Reference: APSB 811

To appear in: *Acta Pharmaceutica Sinica B*

Received Date: 26 March 2020

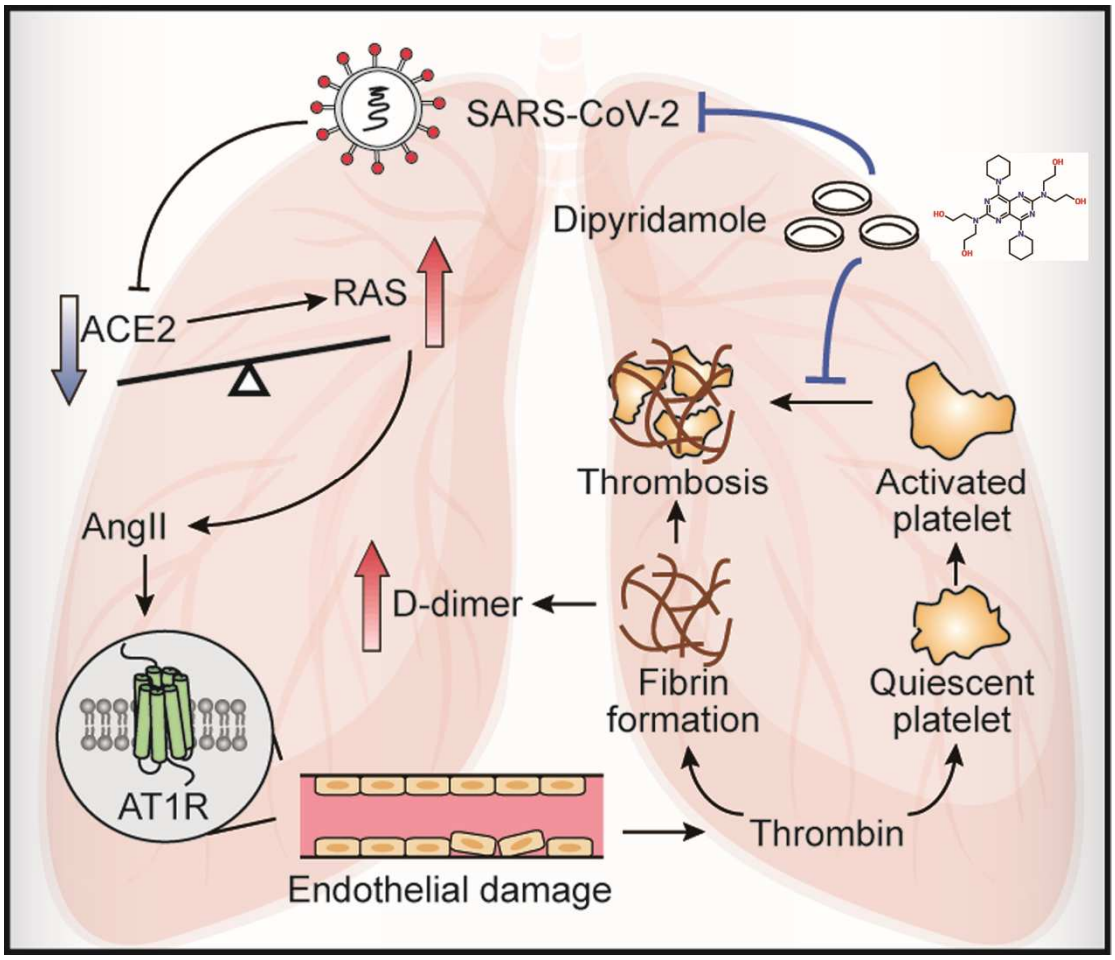
Revised Date: 3 April 2020

Accepted Date: 14 April 2020

Please cite this article as: Liu X, Li Z, Liu S, Sun J, Chen Z, Jiang M, Zhang Q, Wei Y, Wang X, Huang Y-Y, Shi Y, Xu Y, Xian H, Bai F, Ou C, Xiong B, Lew AM, Cui J, Fang R, Huang H, Zhao J, Hong X, Zhang Y, Zhou F, Luo H-B, Potential therapeutic effects of dipyridamole in the severely ill patients with COVID-19, *Acta Pharmaceutica Sinica B*, https://doi.org/10.1016/j.apsb.2020.04.008.

Graphical abstract

Dipyridamole bound to the SARS-CoV-2 protease Mpro after identified *via* the virtual screening and bioassay validation, and thus suppressed viral replication *in vitro*. As a result, dipyridamole supplementation was associated with significantly decreased concentrations of D-dimers, increased lymphocyte and platelet recovery in the circulation, and markedly improved clinical outcomes in comparison to the control patients.



Original article

Potential therapeutic effects of dipyridamole in the severely ill patients with COVID-19

Xiaoyan Liu[a,†], Zhe Li[b,†], Shuai Liu[a,c,†], Jing Sun[d,†], Zhanghua Chen[e,f,†], Min Jiang[g,†], Qingling Zhang[d,†], Yinghua Wei[g], Xin Wang[h], Yi-You Huang[b], Yinyi Shi[c], Yanhui Xu[e], Huifang Xian[e], Fan Bai[f], Changxing Ou[d], Bei Xiong[a], Andrew M. Lew[i], Jun Cui[j], Rongli Fang[e], Hui Huang[k], Jincun Zhao[d,*], Xuechuan Hong[l,m,*], Yuxia Zhang[e,*], Fuling Zhou[a,*], Hai-Bin Luo[b,*]

[a]*Department of Hematology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China*
[b]*Guangdong Provincial Key Laboratory of New Drug Design and Evaluation, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China*
[c]*Dawu County People's Hospital, Xiaogan 432826, China*
[d]*State Key Laboratory of Respiratory Diseases, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, China*
[e]*Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, State Key Laboratory of Respiratory Diseases, Guangzhou Medical University, Guangzhou 510623, China*
[f]*Biomedical Pioneering Innovation Center (BIOPIC), School of Life Sciences, Peking University, Beijing 100871, China*
[g]*Department of Infectious Disease and Department of Pediatrics, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, China*
[h]*Center for Innovative Marine Drug Screening & Evaluation (QNLM), School of Medicine and Pharmacy, Ocean University of China, Qingdao 266100, China*
[i]*Walter and Eliza Hall Institute of Medical Research and Department of Microbiology & Immunology, University of Melbourne, Parkville, Vic 3052, Australia*
[j]*School of Life Sciences, Sun Yat-sen University, Guangzhou 510006, China*
[k]*Cardiovascular Department, the Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen 518000, China*
[l]*State Key Laboratory of Virology, College of Science, Innovation Center for Traditional Tibetan Medicine Modernization and Quality Control, Medical College, Tibet University, Lhasa 850000, China*
[m]*Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (MOE), Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals, Wuhan University School of Pharmaceutical Sciences, Wuhan 430071, China*

Received 26 March 2020; received in revised form 3 April 2020; accepted 6 April 2020

[*]Corresponding authors.
E-mail addresses: luohb77@mail.sysu.edu.cn (Hai-Bin Luo), zhoufuling@whu.edu.cn (Fuling Zhou), yuxia.zhang@gwcmc.org (Yuxia Zhang), zhaojincun@gird.cn (Jincun Zhao), xhy78@whu.edu.cn (Xuechuan Hong).
[†]These authors made equal contributions to this work.

Abstract Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can cause acute respiratory distress syndrome, hypercoagulability, hypertension, and multiorgan dysfunction. Effective antivirals with safe clinical profile are urgently needed to improve the overall prognosis. In an analysis of a randomly collected cohort of 124 patients with Corona Virus Disease 2019 (COVID-19), we found that hypercoagulability as indicated by elevated concentrations of D-dimers was associated with disease severity. By virtual screening of a U.S. Food and Drug Administration (FDA) approved drug library, we identified an anticoagulation agent dipyridamole (DIP) *in silico*, which suppressed SARS-CoV-2 replication *in vitro*. In a proof-of-concept trial involving 31 patients with COVID-19, DIP supplementation was associated with significantly decreased concentrations of D-dimers (P<0.05), increased lymphocyte and platelet recovery in the circulation, and markedly improved clinical outcomes in comparison to the control patients. In particular, all 8 of the DIP-treated severely ill patients showed remarkable improvement: 7 patients (87.5%) achieved clinical cure and were discharged from the hospitals while the remaining 1 patient (12.5%) was in clinical remission.

KEY WORDS Dipyridamole; SARS-CoV-2; COVID-19; Treatment; D-dimer; Severe cases

1. Introduction

As of April 3, 2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, formerly known as 2019-nCoV)[1,2] had infected over 1,000,000 patients in 200 countries, such as USA, Spain, Italy, Germany, France, and UK; this rapid spread has been declared a global pandemic. To date, no agents have been reported to be specific to treat severely ill patents. Identification of readily available drugs for repositioning in Corona Virus Disease 2019 (COVID-19) therapy avails a relatively rapid way to clinical treatment[3].

SARS-CoV-2, together with SARS-CoV and MERS-CoV, belongs to the *beta-coronavirus* genus, which is an enveloped, positive-stranded RNA virus with approximately 30,000 nucleotides[4,5]. Angiotensin I converting enzyme 2 (ACE2) is the receptor that engages the Spike surface glycoprotein of SARS-CoV and SARS-CoV-2[6,7]. ACE2 is highly expressed in many organs, including the lung, heart, kidney, and intestine. Notably, in experimental models of SARS-CoV infection, Spike protein engagement decreases ACE2 expression and activates the renin-angiotensin system (RAS)[6]. RAS activation promotes platelet adhesion and aggregation, and increases the risk for pulmonary embolism, hypertension and fibrosis[8-11]. It also accelerates cardiac and kidney injury by increasing local angiotensin II concentrations[12-14]. Apart from affecting the classic RAS pathway, ACE2 deficiency in the intestine is associated with malnutrition and colonic inflammation[15].

Infection from SARS-CoV can result in severe lymphopenia, prolonged coagulation profiles, lethal acute respiratory distress syndrome (ARDS), watery diarrhea, cardiac disease, and sudden death[9,16-18]. Many features have also been reported for COVID-19, such as prolonged coagulation profiles, elevated concentrations of D-dimers, severe lymphopenia, ARDS, hypertension, and acute heart injury in ICU-admitted patients[2,19]. Given that angiotensin II concentrations were highly elevated in the SARS-CoV-2 infected patients[20], RAS was likely a major pathogenic contributor of disease progression. Indeed, in a recent study describing 1099 patients with COVID-19, the concentrations of D-dimers were elevated in 40% and 60% of the non-severe and severe cases at hospital admission[21], respectively. Furthermore, Zhou et al.[22] showed that a concentration of D-dimer greater than 1 mg/L on admission was associated with significantly increased risk of mortality for patients with COVID-19. Thus, prophylactic anti-coagulation therapy should be considered for alleviating the multi-organ damage for patients with COVID-19.

After viral entry to the host cells, the coronavirus messenger RNA is first translated to yield the polyproteins, which are subsequently cleaved by two viral proteinases, 3C-like protease (3CLP, aka nsp5 or Mpro) and papain-like protease (PLP, or nsp3), to yield non-structural proteins essential for viral replication[23]. Inhibitors that suppress the activity of these proteases may inhibit viral replication and offer an avenue for the SARS-CoV-2 therapy.

Dipyridamole (DIP) is an antiplatelet agent and acts as a phosphodiesterase (PDE) inhibitor that increases intracellular cAMP/cGMP[24]. Apart from the well-known antiplatelet function, DIP may provide potential therapeutic benefits to patients with COVID-19. First, published studies[25-30], including clinical trials conducted in China[31-33], have demonstrated that DIP has a broad spectrum antiviral activity, particularly efficacious against the positive-stranded RNA viruses[26]. Second, it suppresses inflammation and promotes mucosal healing[34]. Third, as a pan-PDE inhibitor, DIP may prevent acute injury and progressive fibrosis of the lung, heart, liver, and kidney[35]. Here we provide evidence advocating DIP as an adjunctive therapy.

2. Results

2.1. DIP suppresses SARS-CoV-2 replication in Vero E6 cells

We virtually screened a U.S. Food and Drug Administration (FDA) approved drug library and found that DIP bound to the SARS-CoV-2 protease Mpro (Fig. 1A and Supporting Information Fig. S1**)**. Hydrophobic and hydrogen bond (H-bond) interactions are the main driving forces for the binding between DIP and Mpro. By free energy perturbation calculations, the binding free energy of ΔG_{pred} was –8.60 kcal/mol with a predicted $IC_{50,\ \text{pred}}$ value of 490 nmol/L by the equation $\Delta G_{\text{pred}}=-RT\ \ln(IC_{50,\ \text{pred}})$. The inhibitory potency of DIP against Mpro was then subjected to an enzymatic assay using a previously published method[36]. As a result, DIP exhibited an $IC_{50,\ \text{exp}}$ value of 530±10 nmol/L

(Fig. 1B), which was consistent with the theoretical prediction of the $IC_{50, pred}$ values.

To directly demonstrate that DIP suppresses SARS-CoV-2 replication *in vitro*, we measured viral titers using a susceptible cell line, the Vero E6 cells. Chloroquine was used as a positive control[37,38]. Remarkably, at concentration 100 nmol/L, DIP suppressed more than 50% of SARS-CoV-2 replication (Fig. 1C). This is four times less than the predicted and experimentally confirmed IC_{50} to suppress Mpro activity, which is consistent with previous findings showing that DIP possesses additional antiviral effects[25-30]. DIP (50 mg oral TID) has been used in patients to prevent hypercoagulability[39], and the serum drug concentration was reported to be around 3 μmol/L[40]. Collectively, these data suggest that the therapeutic dosages of DIP used to treat hypercoagulability could potentially suppress SARS-CoV-2 replication in the infected patients.

Insert Fig. 1

2.2. Demographics and baseline characteristics of the study participants

We first retrospectively analyzed a randomly collected cohort of 124 patients with COVID-19. This has revealed that decreased lymphocyte counts, increased concentrations of D-dimers, CRP, and IL-6 concentrations were significantly associated with disease severity (Table 1).

Insert Table 1

To evaluate the therapeutic potential of DIP as an adjunctive therapy to promote virus clearance and reduce the risk of hypercoagulability, an open label clinical study involving 31 patients was conducted in Dawu County People's Hospital (1st hospital, Xiaogan) and Huangpi Chinese Medicine Hospital (2nd hospital, Wuhan), Hubei province, China from February 3 to March 8, 2020. 12 patients and 10 controls were recruited from the 1st hospital, and 2 patients and 7 controls were recruited from the 2nd hospital. Patients were treated in different isolation wards by different attending physicians. Standard treatment procedures were applied for all patients according to the guidelines formulated by the General Office of National Health Committee. DIP was used in all patients of the selected wards by two specialists each from the two hospitals. Patients from other wards without DIP adjunctive therapy were used as controls.

Baseline characteristics of the two groups were shown in Table 2. The average ages of the patients were 56 years. All patients manifested a cough, >75% had shortness of breath, and 35%–57% had nausea and vomiting. Chest CT scan revealed bilateral pneumonia in the 14 DIP-treated patients and 17 patients in the control group. In addition, RT-PCR test of SARS-CoV-2 RNA was positive for all patients. D-dimer concentrations were elevated in 50% (4/8) and 42% (5/12) of the severely ill patients in the DIP-treated group and the control group, respectively. Comorbidities, including diabetes mellitus, cardiovascular, and cerebrovascular diseases, were found in 6 patients in each of

the DIP and control groups. Patients with diabetes (Table 2) were treated with insulin injections, and those with cardiovascular diseases were treated with nifedipine.

Insert Table 2

2.3. DIP adjunctive therapy increases the clinical cure and remission rates in the severely ill patients with COVID-19

DIP adjunctive therapy was provided in 14 patients. The treatment protocol comprised of 50 mg oral tablets administered thrice daily (a total of 150 mg) for 14 consecutive days. All patients received ribavirin, glucocorticoids, and oxygen therapy, but none received antifungal treatment. Mechanical ventilation was required for all the critically ill patients from the DIP-treated (n = 2), and 1 each from the severely and critically ill patients in the control group (n = 2). Other treatment included antibiotics (42.9% *vs*. 58.8%) and intravenous immunoglobulin (14.3% *vs*. 23.5%).

DIP adjunctive therapy was associated with markedly improved clinical cure and remission rates in both the non-severe and severely ill patients (odds ratio 23.75, P = 0.06; Tables 3 and 4). In particular, for the 8 severely ill patients in the DIP-treated group, 7 patients (87.5%) achieved clinical cure and were discharged from the hospitals, and the remaining 1 patient (12.5%) was in clinical remission. In contrast, for the 12 severely ill patients in the control group, 4 patients (33.3%) were discharged, 2 patients (16.7%) were in remission, and 2 patients (16.7%) died, respectively.

Insert Table 3 and 4

It should be mentioned that due to the urgent situation and the lack of resources to perform viral RNA detection by the participating hospitals, we were unable to accurately determine the effects of DIP to viral clearance. However, according to the qualitative RT-PCR result of SARS-CoV-2 RNA provided by local Centers for Disease Control and Prevention, the average time for virus clearance was shortened by 1.6 days for the severe cases in the DIP-treated group in comparison to the control group.

2.4. DIP adjunctive therapy improves the coagulation profiles and promotes immune cell recovery in the severely ill patients

In analysis of the laboratory indices, we observed continuously increased, albeit not statistically significant, counts of lymphocyte and platelet in patients receiving DIP treatment in comparison to the control patients (Fig. 2). Given that lymphocytopenia and thrombocytopenia are markers of disease severity for patients with COVID-19[20], immune recovery may contribute to infection resolution in DIP-treated patients. It should be noted that 50% and 42% of the severely ill patients from the DIP-treated and control group had increased baseline concentrations of D-dimer, respectively (Table 2). We calculated the dynamic changes for each patient in reference to their own baseline value, and found that D-dimer rose continuously in the control group, whereas they were

decreased in the DIP-treated group (Fig. 2).

Insert Fig. 2

2.5. DIP adjunctive therapy in the two critically ill patients

We also examined two critically ill patients who received DIP adjunctive therapy. A 70-year-old man who had suffered from hypoxia and multiorgan dysfunction at hospital admission unfortunately died 5 days after initiation of DIP treatment. He had an extremely high concentration of D-dimer (16.2 mg/L, Fig. 3A) and a very low lymphocyte count (0.37×10^9/L) at the time of receiving DIP adjunctive therapy. Additionally, his oxygen saturation remained low throughout. In contrast, the other severely ill patient who also had very low oxygen saturation and high D-dimer concentration (8.83 mg/L) at administration had been in clinically remission by the time of manuscript submission. His D-dimer concentration initially increased as high as 15.72 mg/L two days after DIP treatment, but has gradually declined to 2.79 mg/L 4–5 days after DIP adjunctive therapy. This reinforces that high concentrations of D-dimer and low lymphocyte counts are associated with poor prognosis and suggest that DIP treatment should be initiated before the progression to a critical state (Fig. 3B).

Insert Fig. 3

2.6. Chest CT findings with DIP adjunctive therapy

All patients received chest CT scans and showed typical multiple patchy ground-glass shadows in the lungs before the treatment. For the DIP-treated patients, the lesions from all patients had a varied degree of absorption after treatment. In the control group, CT images in 1 of the 12 severely ill patients showed progression (Fig. 4 and Supporting Information Table S1).

Insert Fig. 4

3. Discussion

Despite the enormous threat of SARS-CoV-2, no drugs have been claimed to be specific including the existing drugs used to treat other viruses. In reference to SARS-CoV-2 infection, we hypothesized that the SARS-CoV-2 Spike protein engagement may activate RAS in the lung[6,41]. This hypothesis was supported by published clinical characteristics and biochemical data of the severe and critically ill patients with COVID-19, who showed ARDS, hypertension, acute heart, kidney injury, and positive D-dimer results[2,19,20]. In searching for available anticoagulants, we focused on DIP because of its broad-spectrum antiviral, anti-inflammatory, and anti-fibrotic effects. Very importantly, we found that an EC_{50} value of 100 nmol/L to suppress SARS-CoV-2 replication *in vitro*, indicating that the therapeutic dosage of DIP may potentiate effective antiviral responses in infected patients. These findings are in concordance with our clinical findings of the overall remarkable outcomes in the severely ill patients receiving two weeks of DIP adjunctive therapy. All

the 8 DIP-treated severely ill patients showed remarkable improvement after DIP treatment, with 87.5% discharged from the hospitals and a further 12.5% showing clinical remission. In contrast, for the 12 severely ill patients in the control group, only 33.3% were discharged and death occurred in 16.7%.

In a recent publication describing 1099 patients with COVID-19, D-dimer concentrations were elevated in 40% and 60% of the non-severe and severe cases at hospital admission[21]. It has been reported that a D-dimer concentration greater than 1 mg/L on admission was associated with significantly increased risk of mortality for patients with COVID-19[22]. We found that DIP adjunctive treatment blunted the increase in D-dimer concentrations, and increased the counts of circulating platelets and leucocytes. High concentrations of D-dimers are closely correlated with pulmonary embolism[42], vascular thrombosis, and renal dysfunction[43]. It is a crucial prognostic factor and is important to determine whether ICU-patients recover from severe infections[44,45]. Thus, prophylactic anti-coagulation therapy with DIP should be considered in patients with COVID-19 to reduce the risk of hypercoagulability and multi-organ damage.

It should be mentioned that several factors have limited our ability to fully investigate the therapeutic effects of DIP adjunctive therapy, these include the small number of enrolled patients, the lack of resources to quantify viral replication, and the requirement to follow the treatment guidelines under the circumstances of SARS-CoV-2 outbreak. However, we advocate further trials for DIP adjunctive therapy for patients with COVID-19, particularly for those with early signs of elevated concentrations of D-dimer. DIP has been used world-wide to treat coagulopathy. Additionally, it also exerts anti-inflammatory and antiviral effects in experimental settings and clinical trials. The wide availability, safety, and affordability of DIP argue for further investigation into its therapeutic use in COVID-19, particularly as SARS-CoV-2 infection has been declared a global pandemic.

4. Methods

4.1. Ethics statement

The Ethics Committees from Zhongnan Hospital of Wuhan University (Wuhan, China), Dawu County People's Hospital (Xiaogan, China), and the First Affiliated Hospital of Guangzhou Medical University (Guangzhou, China) approved the study and all patients signed informed consents. Clinical trial (ChiCTR2000030055) was registered.

4.2. Study design

A multicenter parallel randomized controlled clinical trial involving 31 patients was conducted in Dawu County People's Hospital (1st hospital) and Huangpi Chinese Medicine Hospital (2nd hospital) from February 3 to March 8, 2020. We recruited 12 patients and 10 controls from the 1st hospital, and 2 patients and 7 controls from the 2nd hospital. Patients were treated in different isolation wards by

different attending physicians. Standard treatment procedures were applied for all patients according to the guidelines formulated by the Chinese General Office of National Health Committee (Beijing, China). DIP was used in all patients of the selected wards by two specialists each from the two hospitals. Patients from other wards without DIP adjunctive therapy were recruited as controls. Informed written consents were obtained from all patients. The condition of the patients was monitored daily by the attending physicians. Routine laboratory test of the coagulation variables and blood indexes were carried out before, during, and after the treatment. Clinical symptoms and laboratory data were independently validated by two independent investigators for assurance of data accuracy.

4.3. SARS-CoV-2 RNA test by RT-PCR

SARS-CoV-2 RNA from nasopharyngeal swabs were detected upon request of the charging physicians by the local Centers for Disease Control and Prevention (Wuhan, China). Only qualitative data were available for the patients.

4.4. Disease severity assessment

All patients had positive RT-PCR test of SARS-CoV-2 RNA from the nasopharyngeal swab specimens, performed by the local Chinese Center for Disease Prevention and Control. The diagnosis of severe case was made if patients met any of the following criteria: (1) respiratory rate ≥ 30 breaths/min; (2) $SpO_2 \leq 93\%$ while breathing room air; (3) $PaO_2/FiO_2 \leq 300$ mmHg. A critically ill case was diagnosed if any of the following criteria was met: (1) respiratory failure which requiring mechanical ventilation; (2) shock; (3) combined with other organ failure and need to be admitted to ICU.

4.5. Retrospective analysis of the coagulation indices in 124 patients

As of February 8, 2020, 124 confirmed COVID-19 cases had been identified from Zhongnan Hospital of Wuhan University (Table 1). All patients met the diagnostic criteria of "Diagnosis and Treatment Scheme of Novel Coronavirus–Infected Pneumonia (trial 6th)" formulated by the General Office of National Health Committee[46]. A retrospective review of the medical records of these patients was conducted to retrieve coagulation indexes and platelet parameters, including prothrombin time (PT), activated partial thromboplastin time (APTT), plasma fibrinogen (FIB), D-dimer, platelet (PLT) count, and mean platelet volume (MPV). Systemic inflammation was assessed according to the C-reactive protein (CRP), procalcitonin (PCT), and interleukin 6 (IL-6) concentrations.

4.6. Treatment procedures

Anticoagulant therapy was provided *via* oral DIP tablets. The daily treatment protocol comprised of 150 mg in three separate doses for 14 consecutive days. All patients were monitored daily for

possible adverse events. All patients received antiviral (ribavirin, 0.5 g, Q12h), corticoid (methylprednisolone sodium succinate, 40 mg, QID), oxygen therapy, and nutritional support as necessary. Patients with diabetes were treated with insulin injections (Table 2), and those with cardiovascular diseases were treated with nifedipine.

4.7. Free energy perturbation prediction

We virtually screened an U.S. FDA-approved drug database using the SARS-CoV-2 protease Mpro as a drug target. DIP (PubChem CID: 3108, Fig. 1A) was identified as a lead drug. In order to obtain the binding pattern and calculate the binding free energy between DIP and Mpro, DIP was firstly docked onto Mpro by using Glide-SP method with the default parameters[47], and the optimal binding pose (Fig. S1) was further assessed by absolute binding free energy calculation with free energy perturbation[48]. The calculations were carried out in Gromacs 2019[49], and the thermodynamic cycle and procedure was similar to that used by Matteo et al.[50]. In the calculation, the ligand electrostatic and van der Waals interactions were decoupled using a linear alchemical pathway with $\Delta\lambda = 0.10$ for the van der Waals and $\Delta\lambda = 0.20$ for electrostatic interactions. Restraints were added for keeping the relative position between receptor and ligand, which consist of one distance, two angles, and three dihedrals harmonic potentials with a force constant of 10 kcal/mol/Å^2 [rad^2]. The distance and angles for the restraints were determined by the values of the last 2 ns of the 4 ns preliminary MD simulations. In the FEP calculations, 4 ns simulations were performed for each window. The sampled ΔU in the simulations were fitted by Gaussian algorithms and the free energy estimates were obtained by using the Bennet acceptance ratio (BAR) method[51].

4.9. Enzymatic assays of Mpro

The detailed methods of enzymatic assays of Mpro are shown in Supporting Information S1.

4.10. Foci forming assay

Vero E6 cells were seeded in 96-well plates. The cells were pretreated with different dosages of DIP or chloroquine for 1 h before infected with SARS-CoV-2 200 foci forming units (FFU) per well, and overlaid with 1.6% carboxymethylcellulose with different dosages of DIP or chloroquine. After 24 h incubation, cells were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. And then incubated with a rabbit anti-SARS-CoV-2 nucleocapsid protein polyclonal antibody (Sino Biological, Inc., Beijing, China), followed by an HRP-labelled goat anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). The foci were visualized by TrueBlue™ Peroxidase Substrate (KPL, Gaithersburg, MD, USA), and counted with an ELISPOT reader (CTL, Shaker Heights, OH, USA). Viral titers were calculated as FFU per mL.

4.11. Statistical analysis

Statistical analyses and graphics production were performed using R v3.5.3 (Foundation for

Statistical Computing)[52] and GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Categorical variables were described as frequencies or percentages, and continuous variables were shown as mean with standard deviation/error. Comparison for two independent groups was conducted using Student's *t* test (for normally distributed data) or Mann-Whitney test (for non-normally distributed data). Comparison for laboratory indices between the DIP-treatment and control groups during the treatment course was conducted using generalized mixed linear model. Logistic regression was performed to identify factors associated with the clinical outcomes. $P < 0.05$ was considered statistically significant. Detailed descriptions of data comparison and statistical tests were specified in the figure legends.

Acknowledgments

We cordially acknowledge Tencent Cloud and National Supercomputing centers in Guangzhou, Shenzhen, and Tianjin, China for providing HPC resources for virtual screening and free energy perturbation calculations, and Prof. H. Ke at the University of North Carolina, Chapel Hill, NC, USA for comments. We cordially acknowledge National Key R&D Program of China (2017YFB0202600), National Natural Science Foundation of China (91742109, 8152204, 31770978, 81773674, and 21877134), National Health & Medical Research of Australia (1080321, 1143976 and 1150425), Science Foundation of Guangzhou City (201904020023, China), Guangdong Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2016 and 2019, China), Guangdong Provincial Key Laboratory of Construction Foundation (2017B030314030, China), Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT01Y093, China), Zhejiang University special scientific research fund for COVID-19 prevention and control (China), National Health & Medical Research of Australia (1080321, 1143976, and 1150425), Taikang Insurance Group Co., Ltd. and Beijing Taikang Yicai Foundation (Beijing, China), and philanthropy donation from individuals. The funders had no roles in the design and execution of the study.

Author contributions

Jincun Zhao, Xuechuan Hong, Yuxia Zhang, Fuling Zhou, and Hai-Bin Luo co-designed the study and co-led overall data interpretation. Shuai Liu, Xiaoyan Liu, Yinghua Wei, Qingling Zhang, Yinyi Shi, Bei Xiong, and Min Jiang provided patient care and collected clinical data. Zhe Li, Xin Wang, Jun Cui, Hui Huang, and Yi-You Huang performed the virtual screening and enzymatic assay. Jing Sun and Jincun Zhao performed viral suppression assay. Zhanghua Chen, Yuxia Zhang, and Hai-Bin Luo analyzed data and generated the tables and figures. Yuxia Zhang drafted the manuscript with significant input from Andrew M. Lew. All authors interpreted the results and critically revised the

manuscript for scientific content. All authors approved the final version of the article.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Figure 1

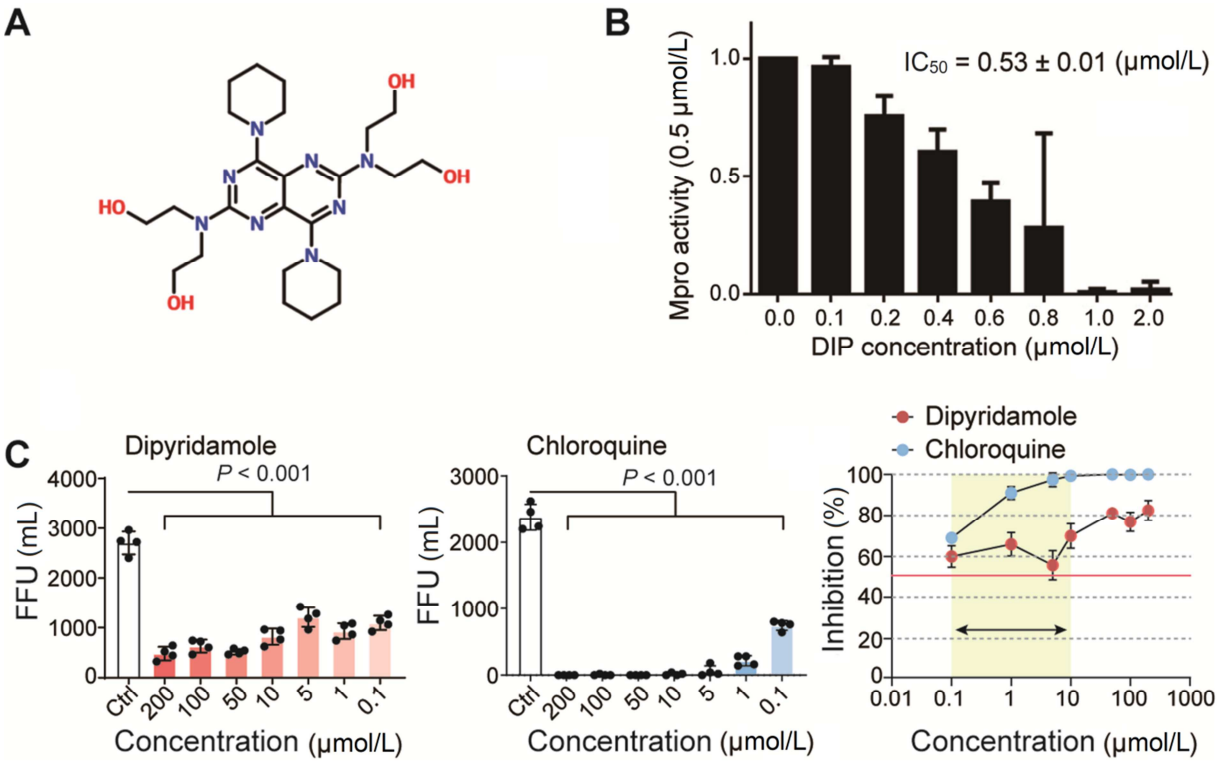


Figure 2

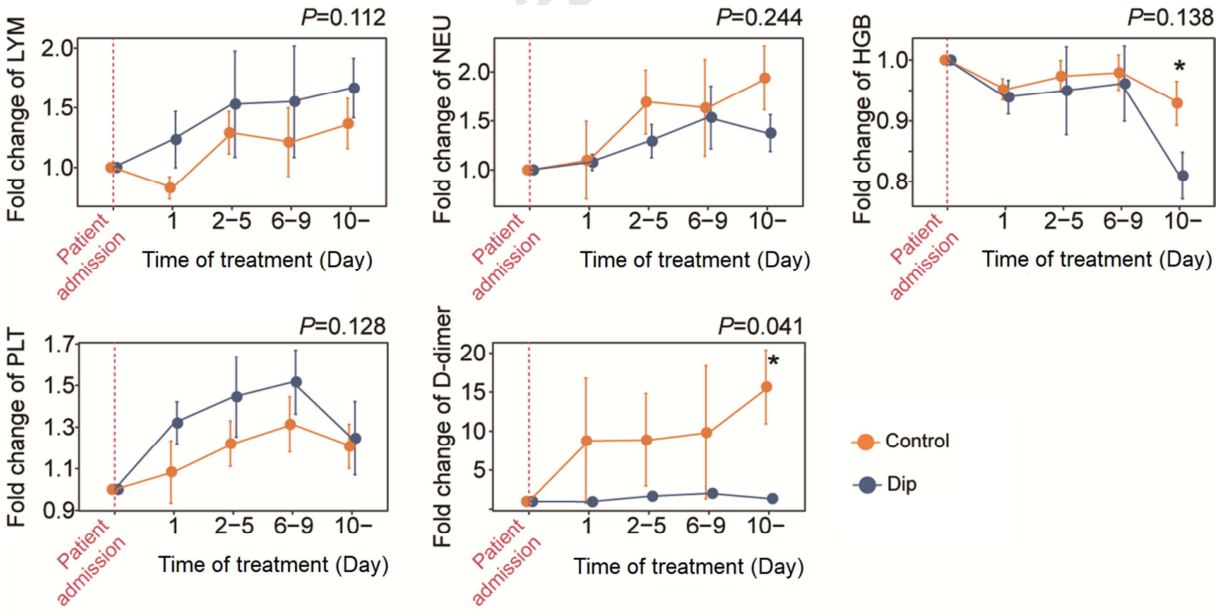


Figure 3

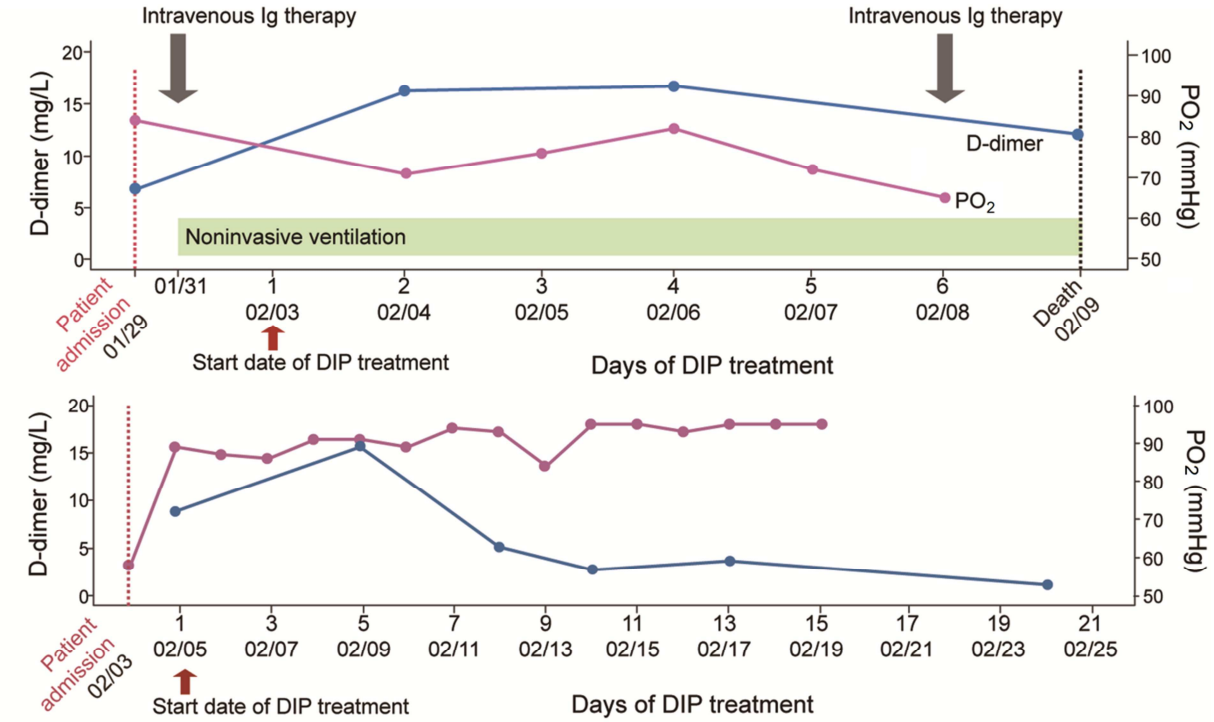
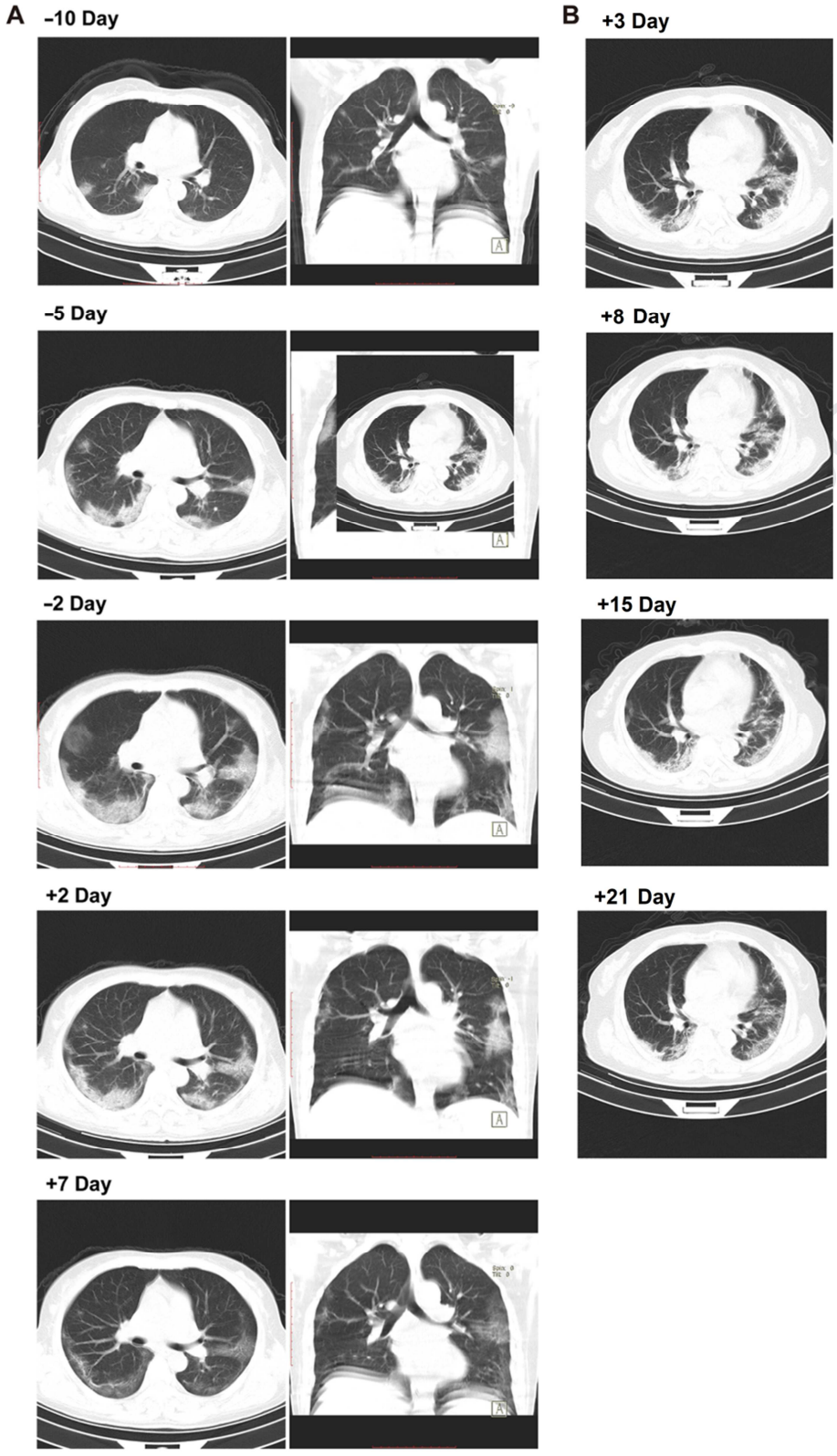
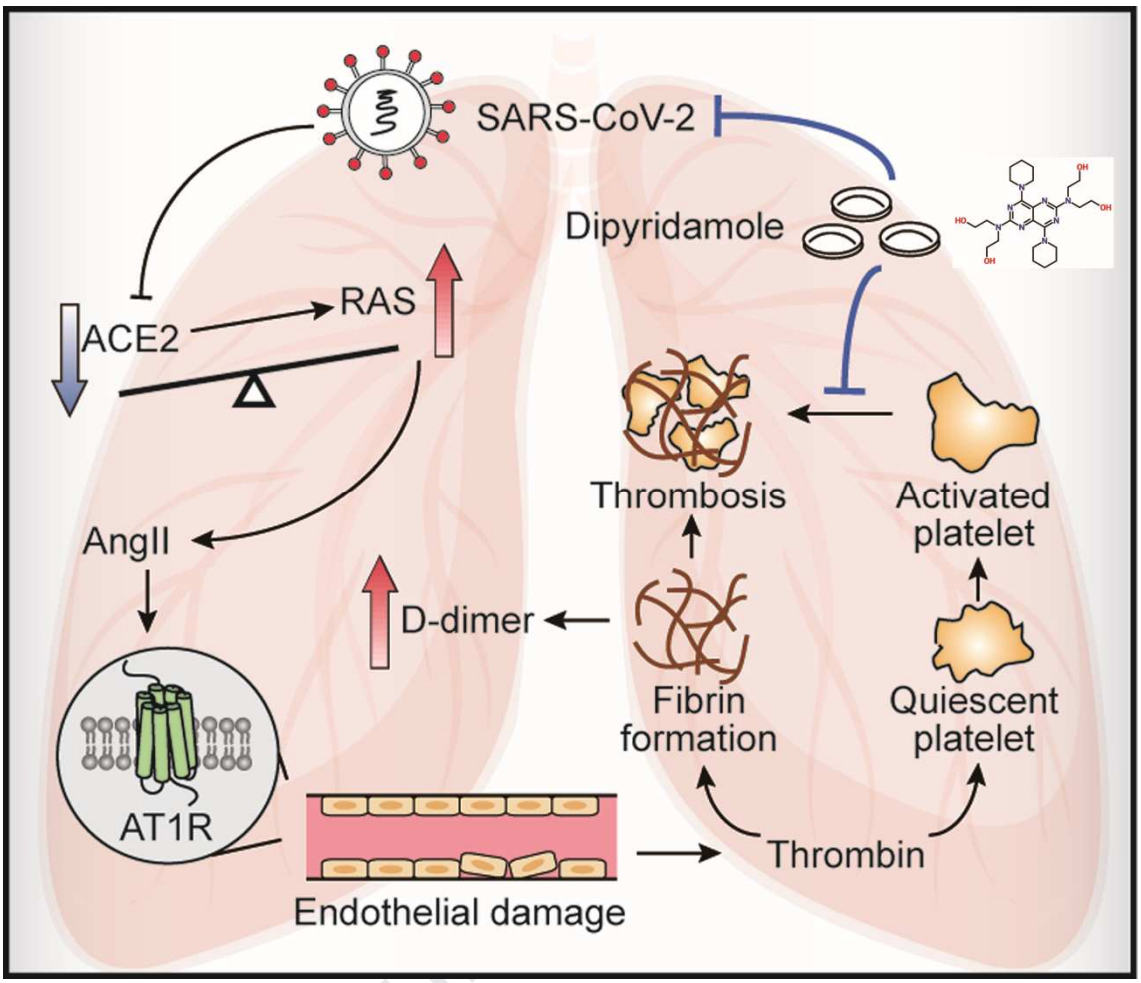


Figure 4



Graphic abstract



Dipyridamole bound to the SARS-CoV-2 protease Mpro after identified *via* the virtual screening and bioassay validation, and thus suppressed viral replication *in vitro*. As a result, dipyridamole supplementation was associated with significantly decreased concentrations of D-dimers, increased lymphocyte and platelet recovery in the circulation, and markedly improved clinical outcomes in comparison to the control patients.

Figure captions

Figure 1 Suppressive effects of dipyridamole (DIP) and chloroquine on SARS-CoV-2 replication *in vitro*. (A) Chemical structure of DIP. (B) Enzyme activity of Mpro in the presence of ascending concentrations of DIP. (C) Dose-dependent suppression of SARS-CoV-2 replication by DIP and chloroquine *in vitro*. Virus titers were measured by Foci forming assay, inhibition rates were performed by indirect immunoinfluscent assay, and calculated inhibition rates of different dosages of DIP or chloroquine were compared with virus control. *P* values were calculated by ANOVA.

Figure 2 Changes of the study variables during treatment. Dynamic changes in the routine blood indexes (lymphocytes, LYM; neutrophils, NEU; hemoglobin, HGB; and platelets, PLT) and coagulation variable (D-dimer) in reference to the baseline values. Data are shown as the means±SD across different time bins during the treatment course. The comparison for each index between the DIP and control groups during the treatment was conducted by generalized mixed linear model. For each specific time bin, comparison for variables between the two groups was conducted using Student's *t* test ($^{*}P < 0.05$).

Figure 3 Changes of D-dimer and oxygen saturation in the two severely ill patients who received DIP treatment. Schematics of the treatment overview and clinical parameters of the deceased critically ill patient (top) and the surviving patient (bottom) who received DIP adjunctive therapy.

Figure 4 Chest CT images in the axial (left panel) and coronal view (right panel) of represented patients with severe COVID-19. (A) Chest CT scans at –10, –5, –2, +2, and +7 day of a patient received DIP treatment. (B) Chest CT scans at +3, +8, +15, and +21 day of a control patient.

Tables

Table 1 Clinical variables in 124 patients with COVID-19.

Variable	Normal range	Non-severe (*n*=87)	Severe (*n*=25)	Critical (*n*=12)	Total (*n*=124)	Total Increased	Total Decreased
		mean±SD (range)				No. (%)	
PLT (10^9/L)	125–350	193.5±70.5 (83–396)	187.6±100.0 (54–525)	187.3±103.7 (85–442)	191.7±80.0 (54–525)	3 (2.4%)	25 (20.2%)
Lymphocyte (10^9/L)	1.1–3.2	1.1±0.3 (0.1–5.0)	0.8±0.3* (0.3–1.7)	0.6±0.4* (0.3–1.4)	0.9±0.6 (0.1–5.0)	–	–
—Decrease no. (%)	–	57 (65.5%)	22 (88.0%)	10 (83.3%)	–	2 (1.6%)	89 (71.8%)
MPV (fL)	6–12	9.1±1.2 (6.6–11.9)	9.1±1.5 (7.3–12.3)	9.4±1.7 (6.6–11.2)	9.1±1.3 (6.6–12.3)	1 (0.8%)	0
PT (S)	9.4–12.5	12.9±1.4 (8.6–17.8)	13.0±1.5 (11.1–17.6)	13.3±1.6 (11.4–15.8)	13.0±1.4 (8.6–17.8)	77 (62.1%)	1 (0.8%)
APTT (S)	25.1–36.5	30.4±3.1 (22.9–38.1)	30.4±2.5 (26.6–34.8)	29.3±4.5 (22.4–35.3)	30.3±3.2 (22.4–38.1)	2 (1.6%)	9 (7.3%)
FIB (mg/dL)	238–498	430.2±80.3 (256–717)	428.9±91.0 (214–582)	428.8±139.4 (203–750)	429.8±88.7 (203–750)	27 (21.8%)	2 (1.6%)
D-dimer (μg/L)	0–500	746.5±2279.7 (59–18825)	1178.4±4267.5 (35–21611)	4138.3±7506.7* (82–26315)	1168.6±3652.7 (35–26315)	–	–
—Increase no. (%)	–	15 (17.2%)	4 (16.0%)	7 (58.3%)	–	26 (21.0%)	–
CRP (mg/L)	0–10	37.0±43.4 (0.4–173.7)	61.5±63.8* (0.7–290.1)	75.0±59.4* (11.6–203.7)	46.0±51.4 (0.4–290.1)	80 (64.5%)	–
PCT (ng/mL)	< 0.05	0.2±0.2 (<0.05–0.65)	0.3±0.3 (<0.05–0.94)	0.2±0.2 (0.07–0.52)	0.2±0.2 (<0.05–0.94)	38 (30.6%)	–
IL-6 (pg/mL)	0–7	39.3±71.1 (2.03–522.2)	55.6±44.4 (2.64–180.5)	81.4±65.6* (7.69–204.3)	48.3±66.5 (2.03–522.2)	79 (63.7%)	–

*$P < 0.05$ when compared to the non-severe group.

–not applicable.

PLT, platelets; MPV, mean platelet volume; PT, prothrombin time; APTT, activated partial prothrombin time; FIB, Fibrinogen; CRP, C reactive protein; PCT, procalcitonin; IL-6, interleukin-6.

Table 2 Baseline characteristics of the 31 enrolled patients.

Variable	Dipyridamole group (*n*=14)	Control group (*n*=17)
General characteristic		
Age (yr)—mean±SD (range)	56±12 (32–74)	56±15 (23–74)
Gender—male no./female no.	8/6	13/4
Group		
Non-severe no./Severe no./Critical no.	4/8/2	3/12/2
Clinical variables		
Cough—no. (%)	14 (100.0%)	17 (100.0%)
Shortness of breath—no. (%)	11 (78.6%)	13 (76.5%)
Nausea and vomiting—no. (%)	8 (57.1%)	6 (35.3%)
Systolic blood pressure (mmHg) —mean±SD (range)	127±12 (120–155) /81±11 (57–124)	128±13 (107–153) /78±10 (56–96)
Partial pressure of oxygen (mmHg) —mean±SD (range)	86±12 (58–93)	92±9 (76–96)
Laboratory values		
Lymphocyte (10^9/L)—mean±SD (range)	1.07±0.57 (0.29–2.28)	0.82±0.45 (0.17–1.85)
Decrease in concentrations of lymphocyte —no. (%)	9 (64.3%)	12 (70.6%)
—no. of non-severe cases (%)	1 /4 (25%)	2/3 (66.7%)
—no. of severe cases (%)	7/8 (87.5%)	9/12 (75%)
—no. of critical cases (%)	1/2 (50%)	1/2 (50%)
D-dimer (mg/L)—mean±SD (range)	2.00±2.54 (0.19–6.84)	1.50±2.73 (0.01–8.43)
Increase in concentrations of D-dimer —no. (%)	6/14 (42.9%)	5/17 (29.4%)
—no. of non-severe cases (%)	1/4 (25%)	0
—no. of severe cases (%)	4/8 (50%)	5/12 (41.7%)
—no. of critical cases (%)	1/2 (50%)	0
Respiratory pathogens		
The nucleic acid of SARS-CoV-2—no. (%)	14 (100.0%)	17 (100.0%)
Unilateral pneumonia—no. (%)	0	1 (5.9%)
Bilateral pneumonia—no. (%)	14 (100.0%)	16 (94.1%)
Comorbidities		
Diabetes mellitus—no. (%)	3 (21.4%)	1 (5.9%)
Cardiovascular disease—no. (%)	2 (14.3%)	3 (17.6%)
Cerebrovascular disease—no. (%)	1 (7.1%)	2 (11.8%)

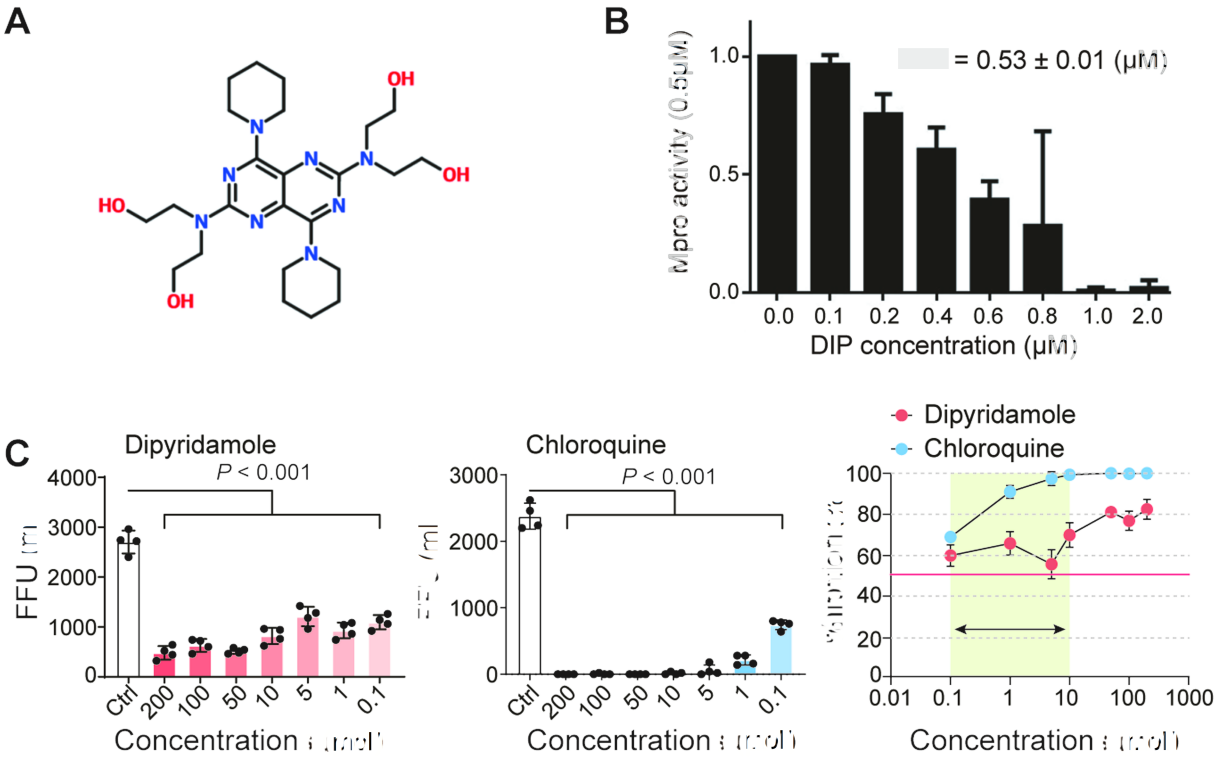
Table 3 Treatment and clinical outcomes of 31 enrolled patients.

Variable	Dipyridamole group (*n*=14)	Control group (*n*=17)
Group		
—Non-severe no./Severe no./Critical no.	4/8/2	3/12/2
Treatment		
Oxygen therapy—no. (%)	14 (100.0%)	17 (100.0%)
Mechanical ventilation—no. (%)	2 (14.3%)	2 (11.8%)
—no. of non-severe cases (%)	0	0
—no. of severe cases (%)	0	1/12 (8.3%)
—no. of critical cases (%)	2/2(100%)	1/2 (50%)
Antibiotic treatment—no. (%)	6 (42.9%)	10 (58.8%)
Antifungal treatment—no. (%)	0	0
Antiviral treatment—no. (%)	14 (100.0%)	17 (100.0%)
Glucocorticoids—no. (%)	14 (100.0%)	17 (100.0%)
Outcome		
Discharge rate—no. (%)	11/14 (78.6%)	7/17 (41.2%)
—no. of non-severe cases (%)	4/4 (100%)	3/3 (100%)
—no. of severe cases (%)	7/8 (87.5%)	4/12 (33.3%)
—no. of critical cases (%)	0	0
Average time for viral clearance (days)	–	–
—severe cases (%)	15.4	17.0
Remission rate—no. (%)	2/14 (14.3%)	2/17 (11.8%)
—no. of severe cases (%)	1/8 (12.5%)	2/12 (16.7%)
—no. of critical cases (%)	1/2 (50%)	0
Progression rate—no. (%)	0	1 /17 (5.9%)
—no. of non-severe cases (%)	0	0
—no. of severe cases (%)	0	1/12 (8.3%)
Death rate—no. (%)	1/14 (7.1%)	4/17 (23.5%)
—no. of severe cases (%)	0	2/12 (8.3%)
—no. of critical cases (%)	1/2 (50%)	2/2 (100%)

–Nonavailable.

Table 4 Multivariate analyses of clinical outcome associated factors.

Variable	Age	Gender (M *vs.* F)	Dipyridamole (Yes *vs.* No)	Ventilation (Yes *vs.* No)	Antibiotic (Yes *vs.* No)	IVIG (Yes *vs.* No)
Coefficients	0.01	0.81	3.17	–20.45	–3.59	0.86
Odd ratio	1.01	2.24	23.75	0	0.03	2.37
95% CI	0.92–1.11	0.09–55.44	0.87–648	0–Inf	0–0.59	0.09–65.08
P value	0.918	0.623	0.06	0.995	0.022	0.609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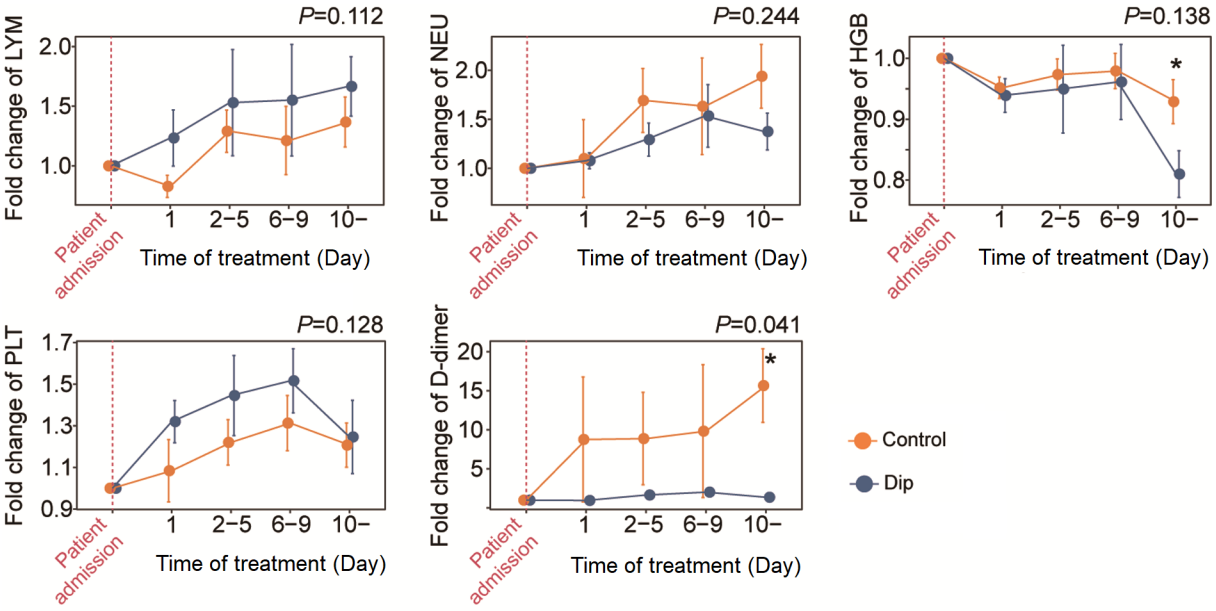

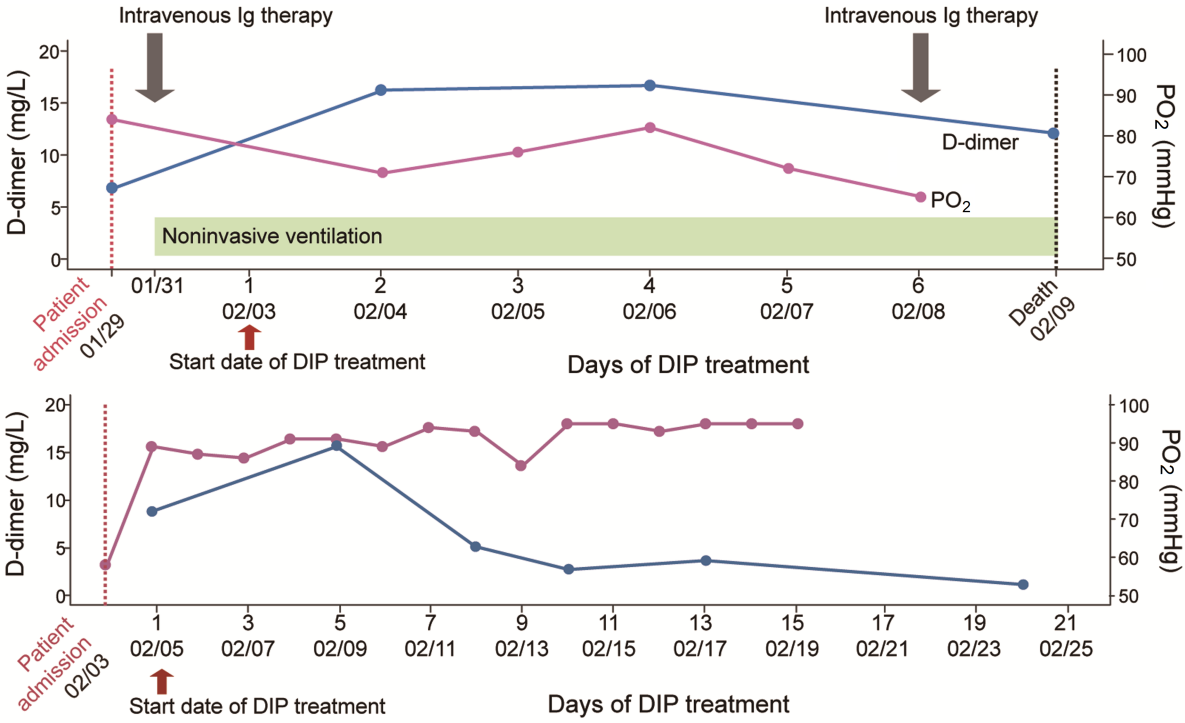

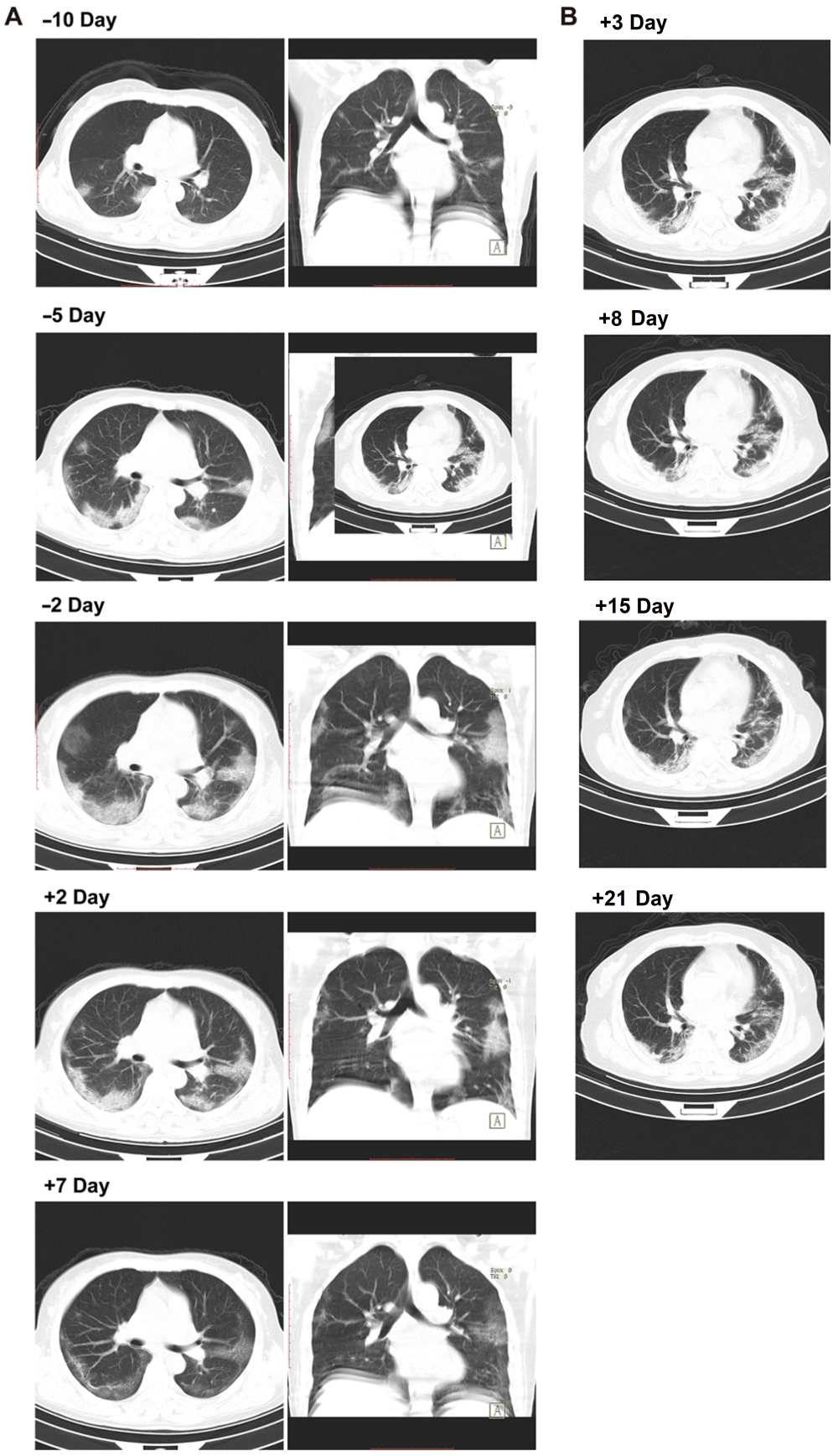
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